IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Art Unit	: 1638	
Examiner	: Anne R. Kubelik	
Serial No.	: 09/807,723	
Filed	: April 18, 2001	
Inventors	: Henry Daniell	
	: William Moar	
Title	: MULTIPLE GENE EXPRESSION FOR ENGINEERING NOVEL PATHWAYS AND HYPEREXPRESSION OF FOREIGN PROTEINS IN PLANTS	Docket No.: 1464-PCT-US-00
		Confirmation No.: 4031
		Dated: July 11, 2002

RESPONSE TO ELECTION/RESTRICTION REQUIREMENT

Commissioner for Patents
Washington, DC 20231

Sir:

Applicants respectfully traverse the restriction of the present claims into 19 groups on the basis that the invention is related to a single inventive concept under PCT Rule 13.1. Namely Applicants' invention employs the use of a single promoter to drive polycistrons resulting in equal expression of the genes, thus enabling the expression of entire pathways in the correct proportions in a single transformation event.

In order to ensure completeness of this response, irrespective of the Applicants' traversal, Applicants hereby elect Group I including Claims 1-3, 6, 15-20, and 60 for examination. Applicants further request the cancellation of Claim 14 (Group IX), thus obviating the restriction between Groups I-VIII and X-XIX and Group IX. It is Applicants' position that this PCT application, currently in the National Phase in the United States, complies with the requirement of Unity of Invention, and therefore Applicants assert that there should be no restriction of the aforementioned invention.

Groups I-VIII and X-XIX have been restricted as not relating to a single inventive

concept because, "these inventions do not constitute an advance over the prior art" as disclosed by Maliga et al (US Patent No. 5,877,402). Applicants respectfully disagree with this conclusion. Maliga et al does not teach a promoter driving a multigene operon. The present invention is directed to the processing and translating of multiple gene transcripts expressed from a heterologous promoter. Applicants' invention employs a single promoter to drive polycistrons, resulting in equal levels of expressions of the polycistrons. Maliga et al teaches fusing a *promoterless* uidA coding region with the rbcL 3' UTR.

In Maliga et al, the inventors introduced the exogenous uidA coding region and showed that the terminator to the rbcL 3' UTR is not sufficiently strong, thus producing a read-through from the native rbcL 3' UTR to the exogenous uidA gene. Maliga et al shows a gene having a flawed termination region, which allows a read-through to other genes. However, this read-through is inconsistent. If the coding region is not properly fused to the upstream region there will not be a read-through. Sometimes there is read-through, and sometimes not, resulting in unequal expression of the genes. This can be seen from Fig. 22C of Maliga et al, where it illustrates that the transcripts are produced in differing amounts. Furthermore, the introduction of multiple genes is not necessarily the introduction of an operon. The claimed invention differs in having a consistently transcribed *operon* where genes are under control of a common operator and are transcribed in a 1:1 ratio to produce a polycistronic mRNA.

Finally we ask the Examiner to consider that Maliga et al shows a read-through to *express* a single foreign gene from a single endogenous promoter. Maliga et al teaches introduction of only a single gene. Applicants' invention provides an opportunity to express entire heterologous pathways in a single transformation event. As further evidence of the novelty of Applicants' common feature shared by the claims in this case, Applicants submit herewith copies of pp 9 and 71-74 of the Journal of Nature Biotechnology (Vol. 19, No. 1,

Jan 2001) where this peer-reviewed journal expressed the uniqueness of Applicants' introduction of the entire Bt *cry*2Aa2 operon. Maliga et al does not teach the introduction of multiple genes in a single foreign heterologous operon and therefore, does not show the technical feature, which Applicant asserts provides Unity of Invention in the present case.

Groups I-VI, Groups VII-VIII, Groups X-XI and XIII-XIX, and Group XII have been restricted as not sharing a special technical feature because the "technical feature is disclosed by McBride et al" (US Patent No. 5,545,818). Applicants respectfully traverse this restriction. McBride et al fails to teach a chloroplast transformation vector capable of introducing multiple genes in a multigene operon. McBride et al provides an expression construct having two unrelated (except for activity) genes: the native cry gene and a synthetic Bt gene. There is no indication the vector is to be used to insert an operon rather than a series of individual genes. This insertion will not produce a polycistronic mRNA. Accordingly, McBride et al fails to anticipate the claims of the present invention. In the present invention, the expression of the second gene was essential for crystal formation of the Bt toxin. As shown in *Milestones in Chloroplast Genetic Engineering: an Environmentally Friendly Era in Biotechnology*, Trends in Plant Science, Vol. 2, No. 2, February 2002 (copy enclosed), the use of the second gene in production of the Bt toxin resulted in a substantial increase in the production of toxin (up to 47% of the total soluble protein). McBride et al cannot accomplish this.

Inserting genes into operons is counter to the established teaching in this field. If this approach were to succeed, a polycistron should be transcribed containing endogenous genes and transgenes. Such polycistrons should be correctly processed to monocistrons prior to translation. However, such processing sequences have not been characterized even today. Therefore, one requires the knowledge of intergenic processing sequences in order to insert transgenes within endogenous operons. This is neither known nor obvious. Thus, there is

no indication in the prior art that transgenes can be inserted within chloroplast operons and successfully processed.

Groups X-XI have been restricted because the "technical feature is not special because it is disclosed by Meagher et al" (US Patent No. 5,965,796). Applicants respectfully traverse this assertion. Meagher et al, through nuclear genetic engineering, teaches a *nuclear* modified form of the MerA and MerB genes to transform plants that are resistant to mercury and organomercurials. Meagher et al disclose a multiple-step transformation event to create an organism that expresses both MerA and MerB (Meahger et al Column 4; lines 1-20). Meagher et al does not teach a single transformation event for the simultaneous introduction of MerA and MerB into a plasmid genome. One of the major drawbacks of Meagher et al is that nuclear genetic engineering requires several backcrosses to create a complete pathway that detoxifies mercury and organomercurials [Bizily S, Rugh CC, Meagher RB. *Phytoremediation of hazardous organomercurials by genetically engineered plants*. Nature Biotechnology 18; 213-217(2000)]. This results in variation of expression levels among different transgenic lines, and affects tolerance to different levels of concentrations of organomercurials. In fact, the nuclear modified form of MerA and MerB genes in transgenic plants sometimes exhibited low levels of tolerance to organomercurials (10um) (Bizily et al 2000). Yet another limitation with the use of nuclear transformed plants in-situ is the escape of the foreign genes via pollen [Daniell H. *GM crops: Public perception and scientific solutions*. Trends in Plant Science. 4, 467-469 (1999): Bogorad, L. *Engineering chloroplasts: an alternative site for foreign genes, proteins reactions, and products*. Trends in Biotechnology. 18, 257-263 (2000)]. Applicant submits that as a result of the limitations described above that the technical feature described in Groups X-XI share a special technical feature.

The present invention in chloroplast transformation is unique and neither anticipated nor obviated by any prior teachings. The Examiner's attention is respectfully directed to the publication, *Multigene Engineering: Dawn of an Exciting New Era in Biotechnology,* Current Opinion in Biotechnology, Volume 13, Issue 2 (copy enclosed) and especially the section labeled "Chloroplast Multigene Engineering" on page 2 thereof.

Accordingly, Applicants respectfully request reconsideration of the restriction requirement, cancellation of Claim 14, Group IX, and the examination of remaining claims on the merits.

Respectfully submitted,

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Sir:

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Name of Applicant, Assignee, Applicant's Attorney
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Date: July 11, 2002